# SOME ASPECTS OF THE ACETYLCHOLINE METABOLISM IN THE SYMPATHETIC GANGLION OF THE IRRADIATED ORGANISM

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In an analysis of the disturbances of the neurohumoral regulation of the functions in the irradiated organism, great significance is attributed to processes that occur in the synaptic processes [4]. In view of this, data on the formation and secretion of mediators of neural excitation during the accomplishment of the interneuronal synaptic transmission of impulses are of interest. The formulation of this question is due to numerous data on the disturbance of acetylcholine metabolism in various tissues after the influence of ionizing radiations [1, 6, etc.].

In this work, the object of investigation was the upper cervical sympathetic ganglion, which is a convenient and widely used model for detecting general principles of interneuronal synaptic transmission [8, 10, 11]. A study of the content of free and bound forms of acetylcholine in the tissue of the ganglion before and after stimulation of the free ganglionic fibers of healthy and irradiated animals permitted a judgement both of the formation, accumulation, and secretion of the investigated substance after activation of the ganglion, and of the disturbance of these processes in the irradiated organism.

## EXPERIMENTAL PROCEDURE

The work was conducted on 57 rabbits, weighing 2.5-3.5 kg. A group of 27 rabbits was subjected to total irradiation by the $\gamma$ rays of $Co^{60}$ at a dose of 400 R. Stimulation of the ganglion and its extirpation were performed under intravenous urethan narcosis (5-6 ml/kg, 10% solution). To determine the total content of acetylcholine, the ganglion, after extirpation and weighing, was treated for 30 min with 1 ml of a 10% trichloroacetic acid solution. In the separate determination of the free and bound fractions, the ganglion was preliminarily triturated with 1 ml of physiological saline, with an addition of $1 \cdot 10^{-5}$ eserine. The eluate was used for immediate determination of the free forms, while the bound fractions were extracted from the solid residue with trichloroacetic acid.

A polarographic method of analysis [5], the sensitivity of which is 0.0001 $\mu$g/ml, was used for the quantitative determination of acetylcholine, which made it possible to determine acetylcholine in a ganglion weighing 10-20 mg. The analyses were conducted on the M-103 micropolarograph. The cervical sympathetic nerve was stimulated with sinusoidal current of superthreshold strength (frequency 100 cps) for two minutes. The physiological effect was judged by the reaction of the pupil. In one group of experiments, both ganglia were subjected to stimulation, while in another one of the ganglia was used for control determinations, since it was noted that there are no differences in the acetylcholine content in the right and left ganglia. The results of different variations of the experiments were close; hence they were subjected to a general analysis.

## RESULTS OF THE EXPERIMENTS

The control investigations were conducted on 30 rabbits. At rest, the average total acetylcholine content, determined in 17 ganglia after extraction with trichloroacetic acid, was $3.99 \pm 0.68$ $\mu$g/g. Tetanization of the

*Deceased.

Acetylcholine Content in the Upper Cervical Sympathetic Ganglion of Control and Irradiated Rabbits (in μg/g)

| Control | | | | 1-3 hours after irradiation | | | |
|---|---|---|---|---|---|---|---|
| rabbit no. | free acetylcholine | bound acetylcholine | total | rabbit no. | free acetylcholine | bound acetylcholine | total |
| 13 | 0,05 | 6,34 | 6,39 | 44 | 0,01 | 0,90 | 0,91 |
| 14 | 0,60 | 1,25 | 1,85 | 45 | 0,25 | 0,31 | 0,56 |
| 15 | 0,76 | 4,60 | 5,36 | 46 | 0,01 | 0,20 | 0,21 |
| 16 | 0,05 | 6,25 | 6,30 | 47 | 0,08 | 0,08 | 0,16 |
| 17 | 0,03 | 1,28 | 1,31 | 48 | 0,03 | 0,05 | 0,08 |
| 18 | 0,41 | 4,70 | 5,11 | 49 | 0,15 | 1,00 | 1,15 |
| 19 | 0,40 | 5,00 | 5,40 | 50 | 0,10 | 0,01 | 0,11 |
| $M \pm m$ | 0,32 ±0,11 | 4,19 ±0,74 | 4,51 ±0,75 | $M \pm m$ | 0,09 ±0,03 | 0,37 ±0,14 | 0,46 ±0,14 |

preganglionic fibers for 2 min led to a statistically reliable increase in the total acetylcholine content. In 11 activated ganglia its amount was an average of 14.10 ±1.48 μg/g. Stimulation of the cervical sympathetic nerve induced a pronounced and stable dilation of the pupil.

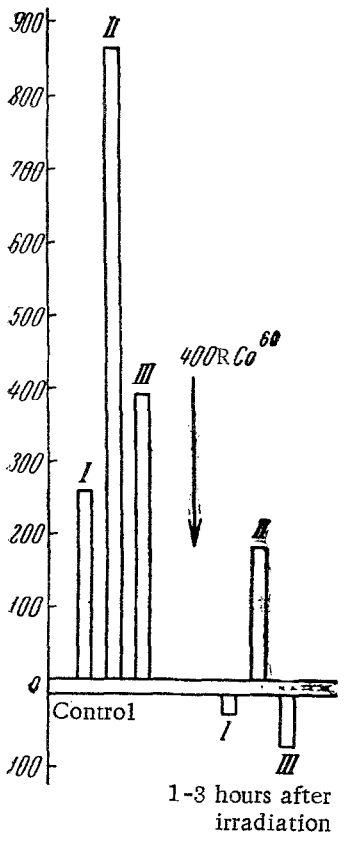
To obtain an idea of the processes of formation, accumulation, and secretion of the mediator, we conducted a special series of experiments with separate determination of the free and bound forms of acetylcholine (see table). In these experiments, the average total acetylcholine content in the control, determined by adding the amounts of free and bound acetylcholine, was 4.51 ±0.75 μg/g. In this case it was found that in unstimulated ganglion, the bulk of the acetylcholine determined (90.7%) exists in the protein-bound state.

The increase in the total acetylcholine after stimulation is due to an increase both in the free and in the bound fractions of it (see figure). The figure presents average data obtained on rabbits in which the right cervical sympathetic nerve was stimulated (the left ganglion served as a control). As can be seen from the figure, the amount of free acetylcholine increased by 865% after stimulation, while that of bound acetylcholine increased by only 33%, which led to an increase in the content of free forms in the stimulated ganglia up to 18% (in comparison with 9.3% at rest).

In the irradiated rabbits, 1.5-24 hours after the influence, substantial changes in the acetylcholine content in the sympathetic ganglion were noted. Moreover, the total amount of acetylcholine in eight animals was increased (49.01 ±21.62 μg/g), while in 19 it was decreased (0.55 ±0.11 μg/g). Considering the changes in the content of each acetylcholine fraction individually, we can establish that the decrease in the total acetylcholine is due primarily to a statistically reliable decrease in the content of its bound forms (see table). As a result of the influence of ionizing radiation, the ratio between the free and bound fractions of acetylcholine in the sympathetic ganglion was changed in the direction of an increase in the free acetylcholine content (up to 20%).

In the case of stimulation of the cervical sympathetic nerve 1-3 hours after irradiation, a picture of changes in the acetylcholine content differing from the norm was observed. In 10 rabbits, two-minute tetanization of the preganglionic fibers either caused no changes in the total acetylcholine content or led to a decrease in its, although the reaction of the pupil was distinct. In an analysis of this phenomenon, it was established that the decrease in the total acetylcholine content in the activated ganglia was due to a decrease in the level of its bound forms (see figure). While the amount of bound acetylcholine dropped after stimulation, the content of the free form increased, as a result of which the level of free forms in activated ganglia of irradiated rabbits reached 69%. Twenty four hours after irradiated, in three rabbits the nature of the reaction to tetanization of the preganglionic fibers remained changed, while in four it was normalized.

Thus, the data obtained indicate a disturbance of the processes associated with the metabolism of the mediator, neural excitation in the sympathetic ganglia of the irradiated organism. Functional inadequacy was especially distinctly manifested in the case of tetanization of the preganglionic fibers. Although in the control the process of




Change in the content of individual fractions of acetylcholine (in % of initial level) in the upper cervical sympathetic ganglion after stimulation of the preganglionic fibers. I) Total acetylcholine; II) free; III) bound.

secretion of the mediator, required for the accomplishment of interneuronal transmission, was accompanied by intensified formation and accumulation of it; in the irradiated animals, in the activation of the ganglion, the level only of free acetylcholine was increased. Moreover, it was secreted in an amount sufficient for the accomplishment of synaptic transmission, which was evidenced by conservation of the pupil reaction. However, during the process of stimulation, no restoration of the reserves of the consumed mediator was noted, as a result of which the total acetylcholine content, and especially that of its bound fractions, proved to be beyond the limits of the initial level.

As for the causes of the disturbance of the process of reservation of acetylcholine by the cholinergic endings, we should consider the influence of ionizing radiation upon the synthesis of this neurohormone and its bonding to protein. The reduction of the amount of bound forms of acetylcholine cannot be the result only of an impairment of its ability to be bonded to protein molecules, since the total content of the mediator in the ganglia line cannot be the result only of an impairment of its ability to be bonded to protein molecules, since the total content of the mediator in the ganglia was reduced in most of the irradiated animals. Probably the influence of ionizing radiation causes some inhibition of the acetylcholine synthesis. Moreover, in the activated ganglia of irradiated animals, the rate of acetylcholine synthesis, in any case by the second minute of the stimulation, remained the same as at rest; there was not that vigorous rearrangement that was observed in the control animals.

The increase in the free acetylcholine content after the influence of ionizing radiation is evidently due to intensive depolarization processes in the presynaptic nerve endings. This can scarcely be attributed to a decrease in the activity of cholinesterase, since the total acetylcholine content in most of the irradiated animals drops sharply, while in the case of inactivation of the enzyme, on the contrary, the ganglion accumulates acetylcholine with an unchanged rate of its liberation [11].

On the basis of the aforementioned, the dynamics of the change in the acetylcholine metabolism in the sympathetic ganglia of irradiated animals is represented in the following way. Originally there is an accumulation of acetylcholine, due to activation of the sympathetic nervous system [3, 4, 7, 9]; this phase is not detected in all animals, since the rapidly developing impairment of the ability of the cholinergic terminals to reserve acetylcholine leads to an exhaustion of the reserves of the mediator. The latter unquestionably is a factor that causes a deterioration of the conditions of synaptic transmission and has a negative influence upon the functional state of the nervous system. This evidently leads to the triggering of compensatory mechanism, one of which may be the previously noted increase in the lability of the ganglionic synapsis [2].

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